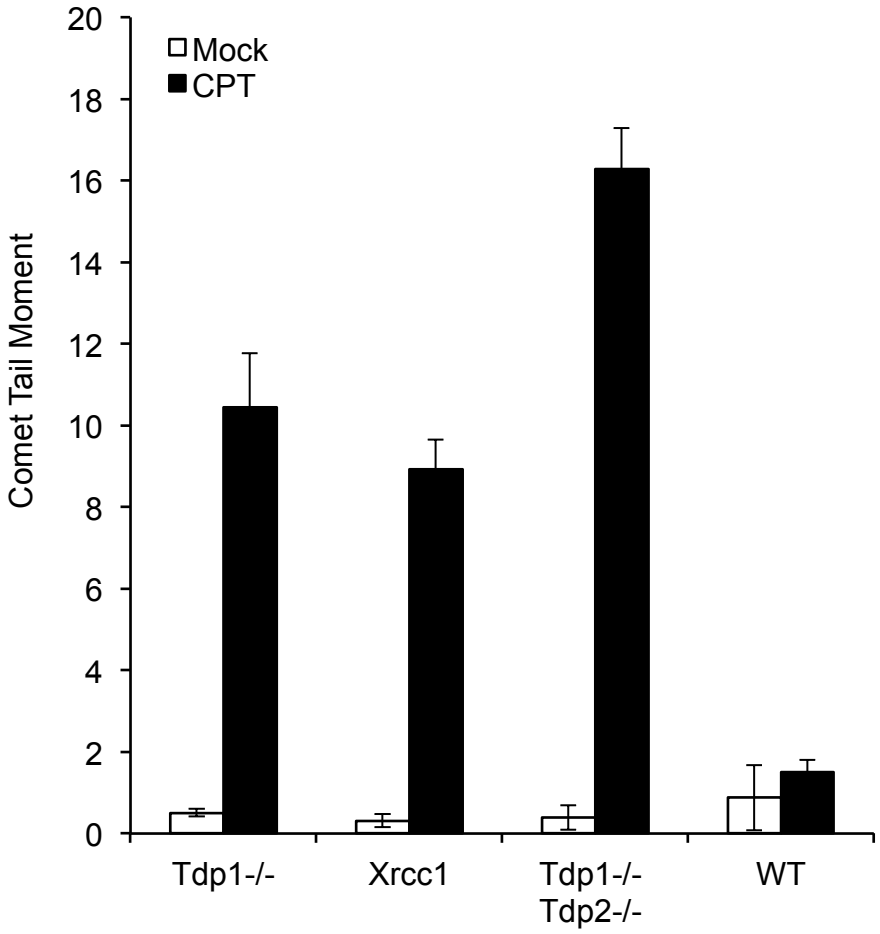
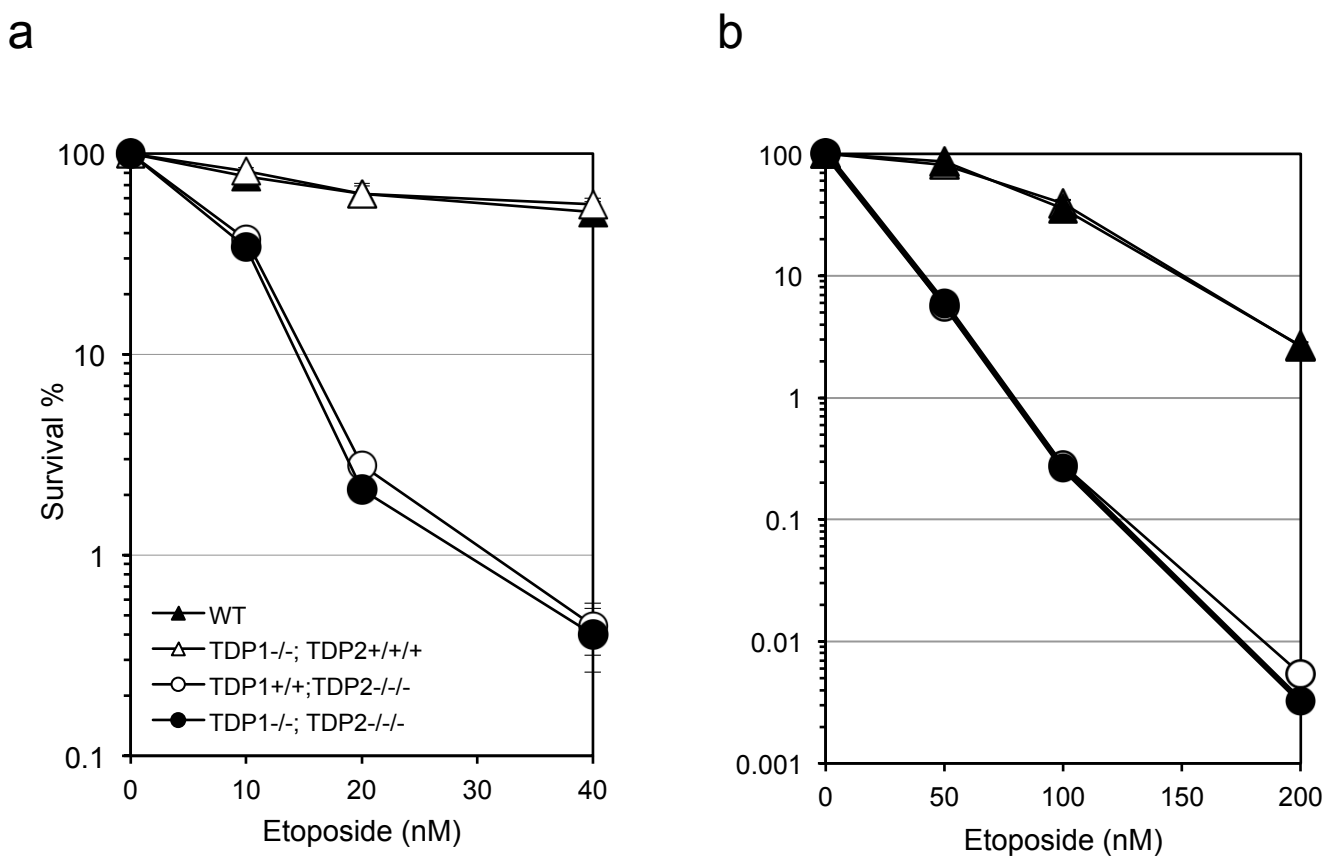
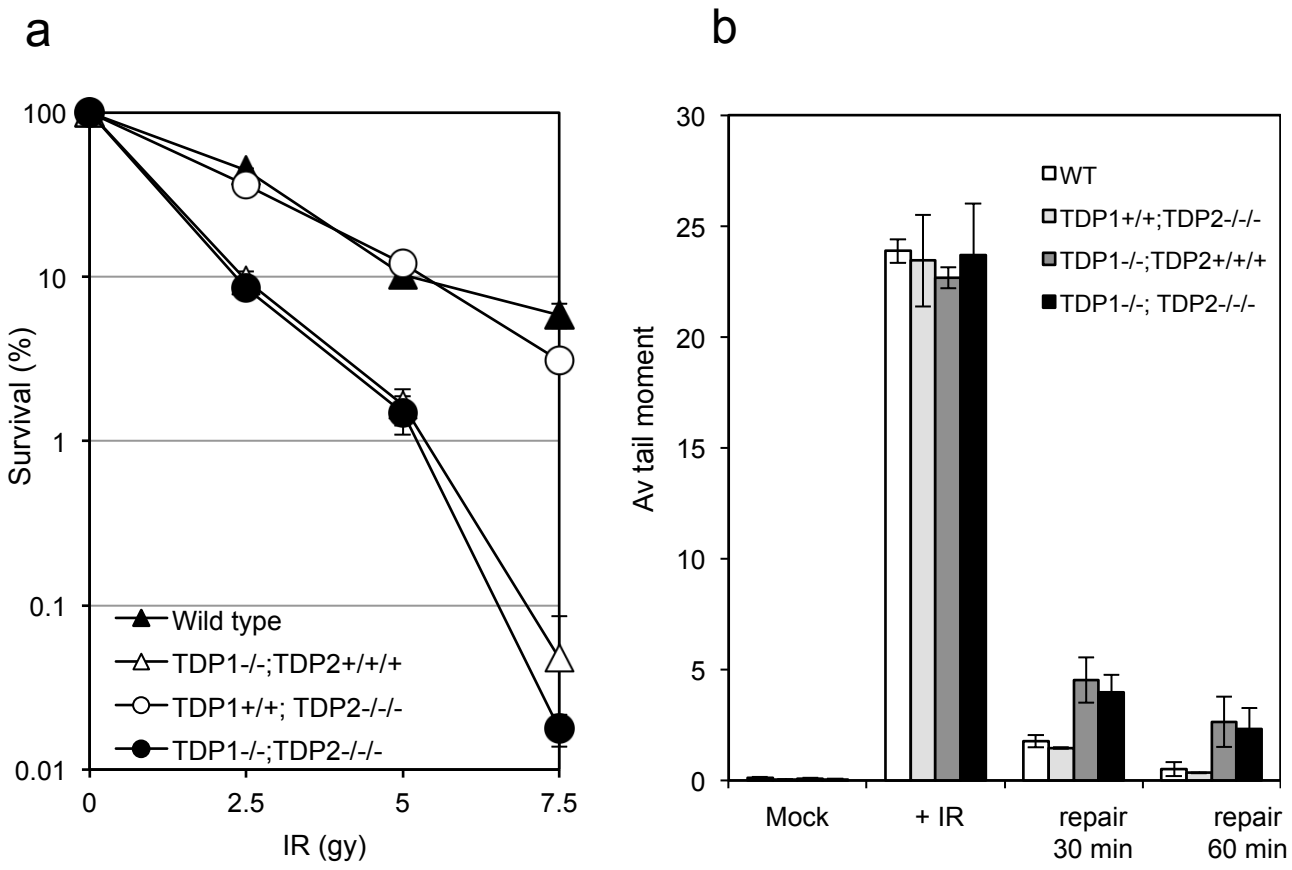


Supplementary Figure 1. Co-deletion of murine *Tdp1* and *Tdp2* results in accumulation of more Top1-mediated DNA damage than deletion of *Tdp1* or *Xrcc1.* MEFs of the indicated genotype were incubated with DMSO or 20μM camptothecin (CPT) for 60 min at 37°C and DNA strand breakage quantified by alkaline comet assays. Mean tail moments were quantified for 50 cells/sample/experiment and data are the average of n=3 biological replicates ± s.e.m.



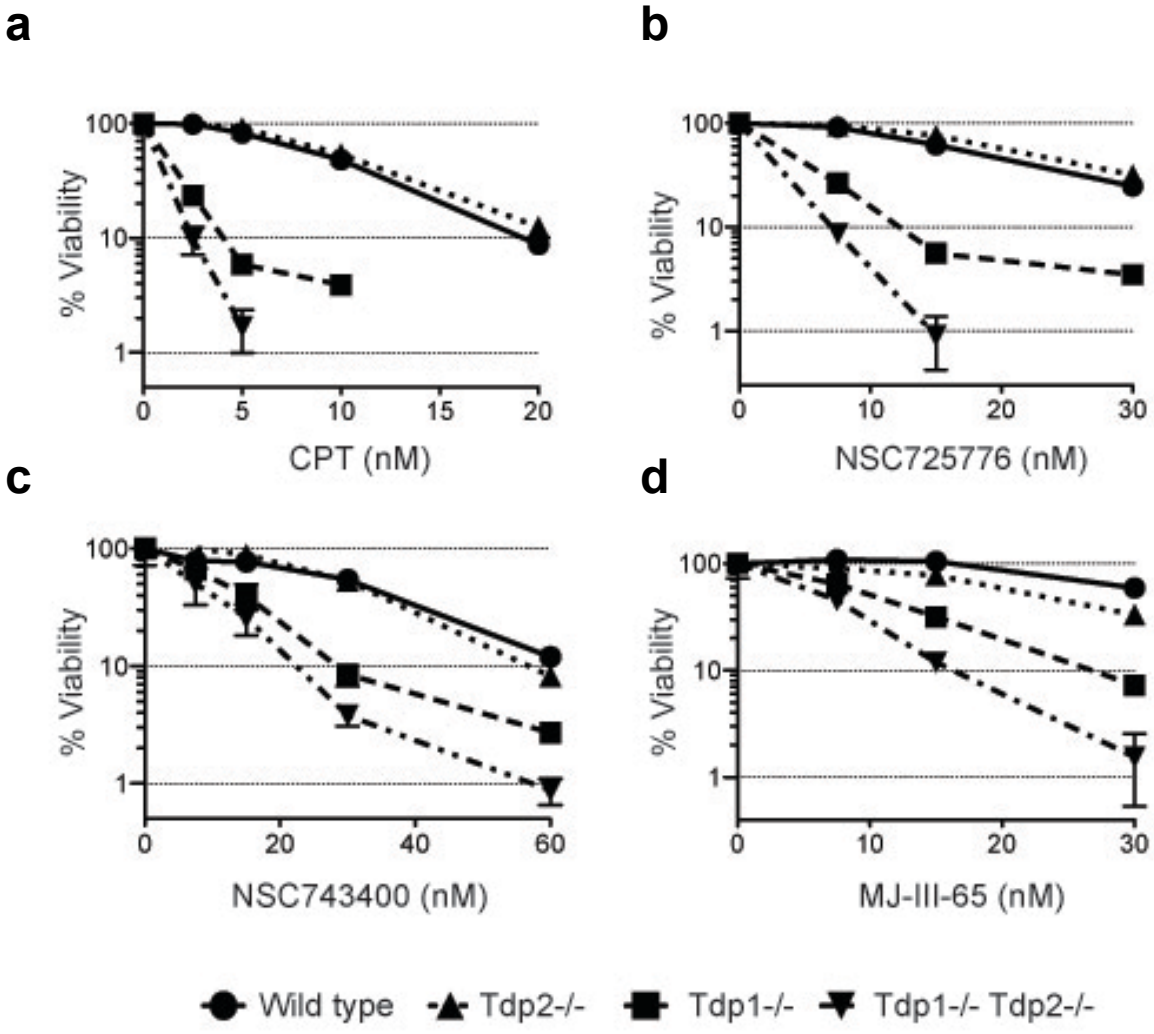
Supplementary Figure 2. Co-deletion of avian *Tdp1* and *Tdp2* do not result in measurable sensitivity to etoposide above that observed for *Tdp2* deletion alone. DT40 cells of the indicated genotype were treated with Etoposide (0-40 nM; **a**) or (0-200 nM; **b**) and the number of surviving colonies was calculated from n=3 biological replicates ± s.e.m

Suppl Fig 3



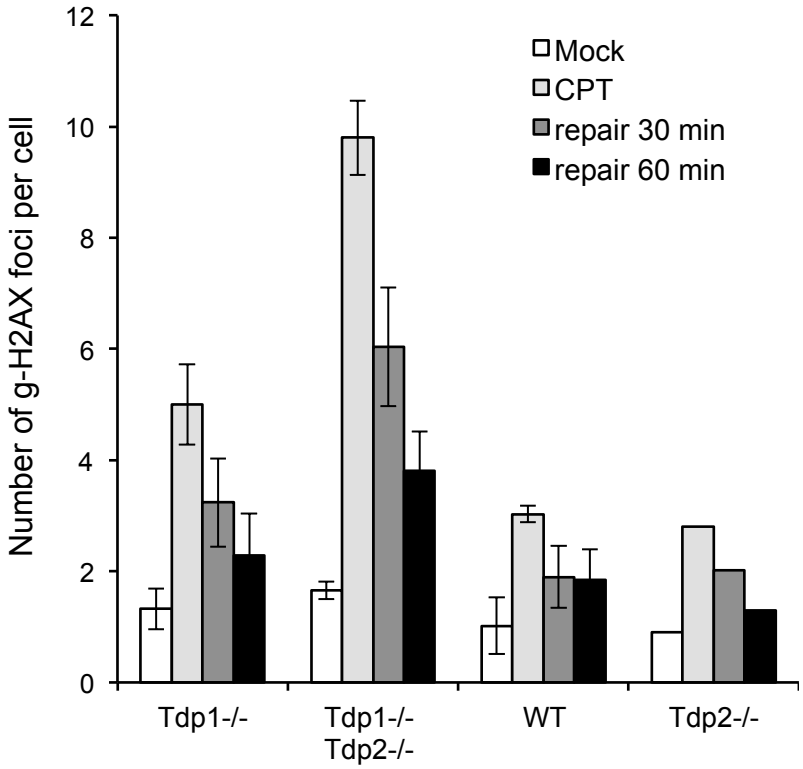
Supplementary Figure 3. Co-deletion of avian *Tdp1* and *Tdp2* do not result in measurable sensitivity to ionizing radiation above that observed for *Tdp1* deletion alone. (**a**) DT40 cells of the indicated genotype were exposed to the indicated doses of X-ray and the number of surviving colonies calculated from n=3 biological replicates ± s.e.m. (**b**) DT40 cells were exposed to 20 Gy ionizing radiation (+IR) and the number of DNA strand breaks quantified during subsequent 30 min or 60 min repair periods by alkaline comet assays. Mean tail moments were quantified for 50 cells/sample/experiment and data are the average of n=3 biological replicates ± s.e.m

Suppl Fig 4



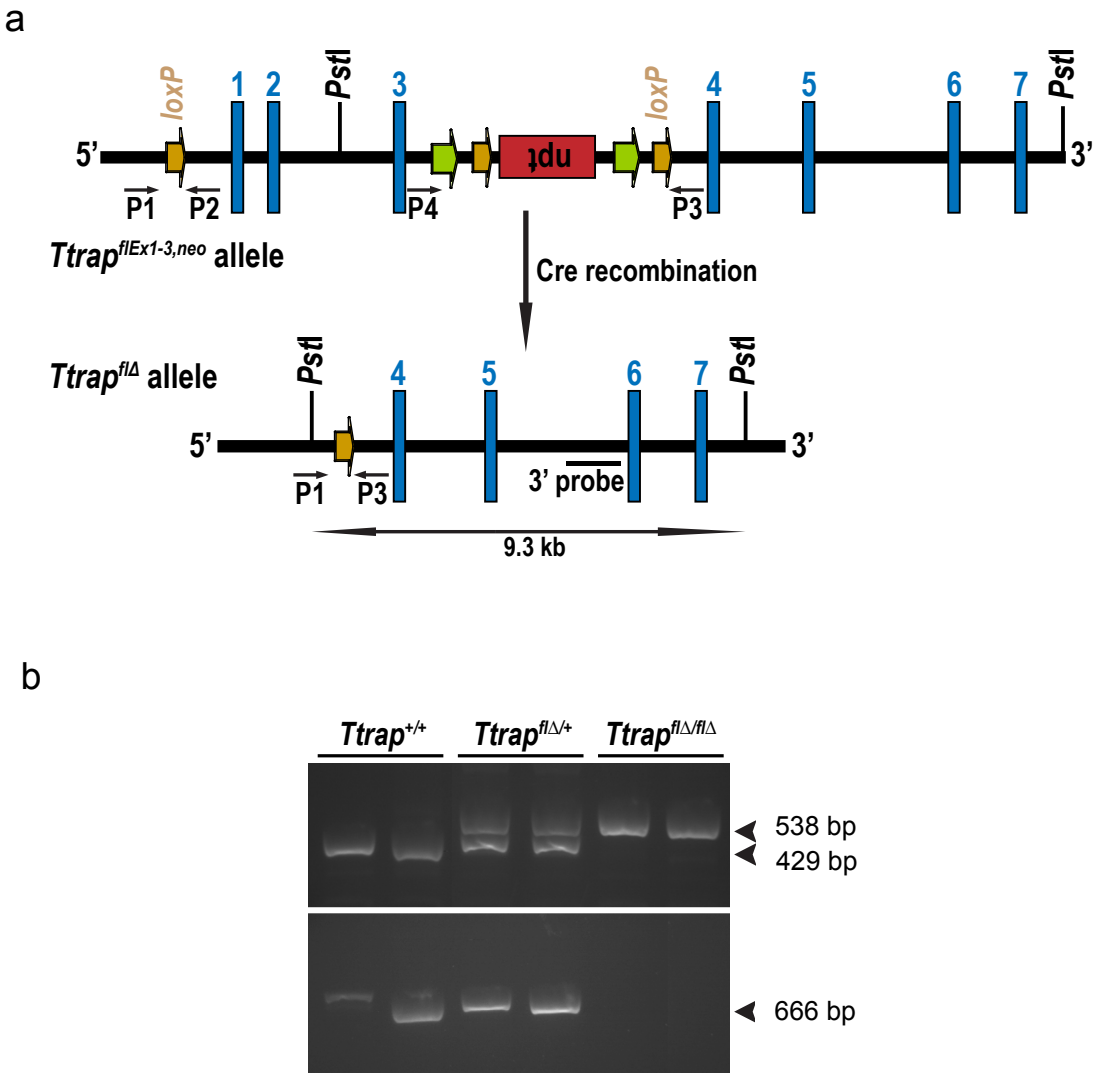
Suppl Fig 4. Avian Tdp2 repairs DNA damage induced by a variety of topoisomerase 1 poisons, in absence of Tdp1. DT40 cells with the indicated genetic background were incubated with the indicated doses of topoisomerase 1 poisons for 72 hours and the number of viable cells was quantified by measuring ATP. Data are the average of n=3 biological replicates ± s.d.

Suppl Fig 5



Supplementary Figure 5. Murine Tdp2 repairs Top1-mediated DNA damage in the absence of Tdp1. MEFs with the indicated genetic background were incubated with 1μM CPT for 30 min at 37˚C "CPT" and subsequently incubated in CPT-free medium for 30 or 60 min "repair 30 min, repair 60 min, respectively". Cells were then fixed and immunostained for g-H2AX. Data are from 50 cells per sample from n=3 biological replicates ± s.e.m.

Suppl Fig 6



Suppl Fig 6. **Generation and confirmation of *Tdp2* (*Ttrap*) knockout mice**. (**a**) Schematic representation of the floxed and the recombined *Ttrap* alleles. Restriction enzyme sites and the corresponding digestion fragments are indicated. Genomic location of primers (P1-P4) used for genotyping are indicated. (**b**) *Upper panel*, PCR analysis on genomic tail DNA from *Tdp2*$^{+/+}$, *Tdp2*$^{+/fl\Delta}$, and *Tdp2*$^{fl\Delta/fl\Delta}$ mice. Primers P1, P2 & P3 were combined in a single PCR reaction to determine the presence or absence of the loxP-flanked sequences and amplified a 429-bp (wild-type allele) or 538-bp (deleted allele) product. *Lower panel*, primers P4-P3 were employed to detect a 666-bp product of *npt (neo)*.

Supplementary Methods

Generation of *Tdp1*$^{-/-}$/*Tdp2*$^{-/-/-}$ DT40 cells

Tdp1$^{-/-}$ and *Tdp2*$^{-/-/-}$ DT40 cells were described previously (Zeng et al., 2011 and Murai et al., 2012). Selection cassettes were excised from the *Tdp2*$^{-/-/-}$ cells by the transient transfection of a Cre recombinase expression vector and addition of 50 nM 5-hydroxytamoxifen. Deletion of the selection cassettes in *Tdp2*$^{-/-/-}$ cells was confirmed by their sensitivity to the appropriate selection drugs. Subsequently, targeting constructs of Tdp1 were transfected, and disruption of *Tdp1* was confirmed by reverse transcription-PCR (RT-PCR).

Drugs

CPT, NSC 743400, NSC 725776, and MJ-III-65 (NSC 706744) were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD, USA). Drug stock solutions were made in DMSO at 10 μM for CPT and 100 μM for NSC 724998, NSC 725776, and MJ-III-65. Stocks were stored at -20ºC and diluted with complete medium.

Cellular viability assays

Cells were continuously exposed for 72 hours to the indicated concentrations of drugs. Approximately 200 DT40 cells were seeded into 384-well white plate (#6007680 Perkin Elmer Life Sciences, Waltham, MA) with 40 μl of medium per well. Cell viability was determined using the ATPlite 1-step kit (PerkinElmer). Briefly, 20 μl for 384-plate ATPlite solution was added to each well. After 5 min, luminescence was measured by Envision 2104 Multilabel Reader (PerkinElmer). The ATP level in treated cells was determined as a fraction of that in untreated cells and presented as % viability.